





**Notes:**

- Prepare TNB Standard just before use as it is easily oxidized. Discard unused Standard.  $\text{TNB } \epsilon = 13600 \text{ m}^{-1} \text{ cm}^{-1}$
  - Since 1 part of DTNB generates 2 parts of TNB, the TNB Standard has been adjusted by factor 2.
- 2. Reaction Mix:** Mix enough reagents for the number of assays to be performed.

**Extracellular PAF-AH:** For each well, prepare 98  $\mu\text{l}$  Reaction Mix containing:

|                     | Reaction Mix     | Background Control Mix* |
|---------------------|------------------|-------------------------|
| PAF-AH Assay Buffer | 97 $\mu\text{l}$ | 99 $\mu\text{l}$        |
| DTNB Probe          | 1 $\mu\text{l}$  | 1 $\mu\text{l}$         |

Mix well by vortexing. Add 98  $\mu\text{l}$  of Reaction Mix to each well containing Positive Control and samples. Mix and incubate at room temperature for 30 min. After incubation, add 2  $\mu\text{l}$  of PAF-AH Substrate into Positive Control and sample wells. Mix well.

\*Add 100  $\mu\text{l}$  of Background Control Mix to sample background control well(s).

**Intracellular PAF-AH:** For each well, prepare 50  $\mu\text{l}$  Reaction Mix containing:

|                     | Reaction Mix     | Background Control Mix |
|---------------------|------------------|------------------------|
| PAF-AH Assay Buffer | 48 $\mu\text{l}$ | 50 $\mu\text{l}$       |
| PAF-AH Substrate    | 2 $\mu\text{l}$  | ---                    |

Mix well and add 50  $\mu\text{l}$  of Reaction Mix to each well containing Positive Control and samples. Incubate at room temperature for 30 min. Prepare mix of 1  $\mu\text{l}$  of DTNB and 49  $\mu\text{l}$  of PAF-AH Assay Buffer for each well. Mix well by vortexing. Make as much as needed, add 50  $\mu\text{l}$  of this mix to Positive Control, background control, and sample wells. Mix well.

**4. Measurement:**

**Extracellular PAF-AH:** Measure absorbance (412 nm) immediately in kinetic mode for 20-60 min. at room temperature.

**Note:** Incubation time depends on the PAF-AH activity in the samples. We recommend measuring OD in kinetic mode, and choosing two time points ( $T_1$  &  $T_2$ ) in the linear range to calculate the PAF-AH activity of the samples. The TNB Standard Curve can be read in Endpoint mode (i.e., at the end of incubation time).

**Intracellular PAF-AH:** Measure absorbance (412 nm) immediately at room temperature (End-point).

**5. Calculation:** Subtract 0 Standard reading from all readings. Plot the TNB Standard Curve. If sample background control reading is significant, subtract sample background control reading from sample reading.

**Extracellular PAF-AH:** Calculate the PAF-AH activity of the test sample:  $\Delta\text{OD} = A_2 - A_1$ . Apply  $\Delta\text{OD}$  to the TNB Standard Curve to get B nmol of TNB generated by PAF-AH during the reaction time ( $\Delta T = T_2 - T_1$ ).

**Intracellular PAF-AH:** Apply corrected OD to the TNB Standard Curve to get B nmol of TNB generated by PAF-AH during the incubation time ( $T = 30 \text{ min}$ ).

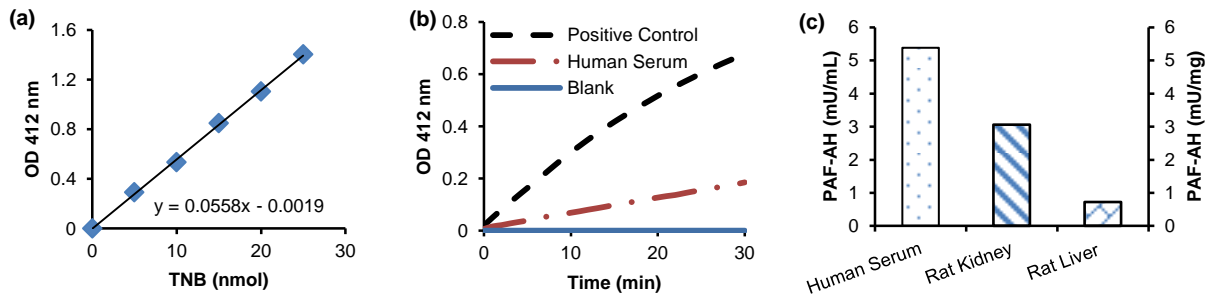
$$\text{Sample PAF-AH Activity} = \frac{B}{(T^* \times V)} \times \text{Dilution Factor} = \text{nmol/min}/\mu\text{l} = \text{mU}/\mu\text{l} = \text{U/ml}$$

Where: **B** is TNB amount in the sample well from Standard Curve (nmol).

**T\*** is reaction time (min.). For extracellular PAF-AH activity, it is  $\Delta T = T_2 - T_1$  and for intracellular PAF-AH activity,  $T = 30 \text{ min}$ .

**V** is sample volume added into the reaction well ( $\mu\text{l}$ ).

**Unit Definition:** One unit of PAF-AH is the amount of enzyme that generates 1.0  $\mu\text{mol}$  of TNB per min. at pH7.2 at 25°C.



**Figure:** (a) TNB Standard Curve. (b) Measurement of PAF-AH activity in human serum. (c) Relative PAF-AH Activity was calculated in human serum (20  $\mu\text{l}$ ) and lysates prepared from rat kidney (75  $\mu\text{g}$ ) and liver (45  $\mu\text{g}$ ). Assay was performed following the kit protocol.

**VIII. RELATED PRODUCTS:**

- Acetylcholinesterase Activity Colorimetric Assay Kit (K764)  
PhosphoSeek™ PI3-Kinase Fluorometric Assay Kit (K706)  
PhosphoSeek™ PTP1B Fluorometric Assay Kit (K707)  
Myeloperoxidase (MPO) Colorimetric Assay Kit (K744)  
MPO Inhibitor Screening Kit (K746)  
Sphingomyelinase Activity Colorimetric Assay Kit (K599)  
Sphingomyelin Quantification Colorimetric Assay Kit (K600)
- Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (K739)  
PhosphoSeek™ PDE5A Fluorometric Assay Kit (K705)  
PhosphoSeek™ Sphingosine Kinase-1 Fluorometric Assay Kit (K708)  
Myeloperoxidase Fluorometric Assay Kit (K745)  
MPO Peroxidation Assay Kit (K747)  
Sphingomyelinase Activity Fluorometric Assay Kit (K574)

**FOR RESEARCH USE ONLY! Not to be used on humans.**